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Purification and properties of an unusual NADPH-dependent ketose reductase from the silverleaf whitefly

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Abstract

Sorbitol accumulates in the silverleaf whitefly when this insect is exposed to elevated temperatures. Synthesis of sorbitol in the silverleaf whitefly is catalyzed by an unconventional enzyme that converts fructose to sorbitol using NADPH as the coenzyme. In the present study, the NADPH-dependent ketose reductase from adult whiteflies was purified to apparent homogeneity and characterized. The NADPH-dependent ketose reductase was tetrameric, composed of 38.7 kD subunits, and catalyzed both fructose reduction and sorbitol oxidation. The purified whitefly enzyme exhibited an almost exclusive requirement for NADP(H) for ketose reduction/sorbitol oxidation. The pH and temperature optima for fructose reduction and sorbitol oxidation were 7 and 45°C and > 9 and 50°C, respectively. The affinity of the enzyme for fructose was very low, but physiological considering the high concentrations of carbohydrate available to this phloem-feeding insect. Edman degradation analysis of three peptides from the enzyme showed that their amino acid sequences matched internal sequences of NAD^+-dependent sorbitol dehydrogenases. Thus, the NADPH-dependent ketose reductase responsible for sorbitol synthesis in the silverleaf whitefly is structurally similar to the sorbitol catabolic enzyme NAD^+-dependent sorbitol dehydrogenase, but differs in its coenzyme requirement. Antibodies directed against the purified whitefly enzyme showed that this novel ketose reductase occurs in whitefly eggs and nymphs, as well as in the adults. Published by Elsevier Science Ltd. All rights reserved

Keywords: Polyol Sorbitol Insect carbohydrates Aldose reductase Sorbitol dehydrogenase

1. Introduction

The silverleaf whitefly, *Bemisia argentifolii* Perring and Bellows (Homoptera: Aleyrodidae), is widely distributed throughout tropical and subtropical regions of the world (Byrne and Bellows, 1991). Whiteflies thrive in arid regions where they can attain almost plague-like populations on cotton, causing severe reductions in yield (Gerling et al., 1980; Henneberry et al., 1995). Previously, we showed that the polyol sorbitol accumulated in whiteflies when they were exposed to elevated temperatures while actively feeding (Wolfe et al., 1998). Since sorbitol was synthesized de novo from recently ingested sucrose, there was no accumulation of sorbitol in non-feeding whiteflies (Wolfe et al., 1998). Based on the observation that elevated temperatures were lethal to non-feeding whiteflies and the well-known ability of polyols to protect enzymes against temperature denaturation (Kim and Lee, 1993; Erarslan, 1995), we proposed that sorbitol functions as a thermoprotectant in the silverleaf whitefly (Wolfe et al., 1998).

Sorbitol is one of the most abundant polyols in nature. In plants, this polyol is synthesized in the phosphorylated form by reduction of glucose-6-P by an NADPH-dependent sorbitol 6-phosphate dehydrogenase (Yamaki, 1980). In animals, sorbitol is synthesized from glucose via an NADPH-dependent aldose reductase (Jeffery and Jörnvall, 1988). Both enzymes are members of the aldo-keto reductase superfamily which includes aldehyde reductase, carbonyl reductase and aldose reductase (Bohren et al., 1989). Catabolism of sorbitol in animals generally involves oxidation to fructose by an NAD^+-dependent sorbitol dehydrogenase (NAD^+-SDH, Jeffery and Jörnvall, 1988). This enzyme is a zinc-containing

Abbreviations: KR = ketose reductase; NADPH-KR = NADPH-dependent ketose reductase; NAD^+-SDH = NAD^+-dependent sorbitol dehydrogenase; SDH = sorbitol dehydrogenase

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protein whose overall structure places it within the medium-chain alcohol dehydrogenase family (Jörnvall et al., 1995).

Extracts prepared from adult whiteflies exhibited considerable NADPH-dependent ketose reductase (NADPH-KR) activity, but no detectable aldose reductase activity. Radiotracer experiments using artificial diets showed that fructose was the immediate precursor to sorbitol (Wolfe et al., 1998). Thus, sorbitol synthesis in the silverleaf whitefly is unconventional, involving an NADPH-dependent enzyme that synthesizes sorbitol from a ketose (i.e., fructose) rather than an aldose (i.e., glucose). In this study, we describe the isolation and characterization of the novel NADPH-KR from the silverleaf whitefly.

2. Materials and methods

2.1. Plant and insect material

Silverleaf whiteflies (*Bemisia argentifolii*, Perring and Bellows) were reared on cotton plants (*Gossypium hirsutum* L., var. Coker 100A glandless) as described previously (Salvucci et al., 1997). For protein purification, approximately 300 g of whiteflies were collected from an infested melon field in Holtville, CA using a gasoline-powered vacuum. Whiteflies were transported from the field on ice, frozen overnight at −80°C and then sieved through two metal screens supported on a 14 mesh (1.18 mm) soil sieve.

2.2. Purification of whitefly KR

All procedures were performed at 4°C. Twenty grams of frozen whiteflies were extracted in a blender containing 200 ml of 50 mM HEPES-KOH, pH 7.8, and 5 mM 2-mercaptoethanol (buffer A). The extract was filtered through four layers of Miracloth and the filtrate was centrifuged for 20 min at 20 000***g***. The supernatant, which contained all of the KR activity, was loaded on a 2.6 × 15 cm Q-Sepharose column. Ketose reductase activity was not retained on the column and was eluted with two column volumes of buffer A. Protein in the eluate was precipitated by addition of solid ammonium sulfate and the fraction precipitating between 25 and 70% (w/w) ammonium sulfate was collected by centrifugation for 12 min at 15 000***g***. The protein pellet was dissolved in 10 ml of 0.1 M potassium phosphate, pH 6.2, 5 mM 2-mercaptoethanol and 5% glycerol (buffer B), and the solution was centrifuged for 12 min at 15 000***g***. Following centrifugation, the solution was chromatographed overnight at 0.4 ml min^{-1} on a 2.6 × 56 cm column of Sephacryl S-300. Fractions containing KR activity were pooled and loaded directly on a 1 × 5 cm column of Matrex Orange (Amicon, Danvers, MA)[1] at 0.5 ml min^{-1}. Since the eluate contained considerable KR activity, it was recycled back through the column. The column was then rinsed with eight column volumes of buffer B before elution of KR with buffer B containing 0.2 mM NADPH. For some preparations, chromatography on Reactive Red (Sigma Chemical Co., St. Louis, MO) was used instead of Matrex Orange. Compared to Matrex Orange, Reactive Red was more efficient at binding KR, but required a 10-fold higher concentration of NADPH for elution. Active fractions from the dye-ligand column were pooled and dialyzed overnight against 20 mM MES-KOH, pH 6.0, and 5 mM 2-mercaptoethanol (buffer C). The dialyzed solution containing KR activity was further fractionated by cation-exchange perfusion chromatography on a 0.46 × 10 cm POROS-SP column (PerSeptive Biosystems, Framingham, MA). The column was equilibrated with buffer C and bound protein was eluted with a 0 to 0.5 M linear gradient of KCl. Fractions containing ketose reductase activity eluted from this column with approximately 0.3 M KCl and were stored at −80°C. There was no detectable loss of activity after several months of storage under these conditions.

2.3. Enzyme assays

Ketose reductase and SDH activities were measured at 30°C by monitoring the decrease or increase in A_{340} upon oxidation or reduction of NADP(H) or NAD(H). Unless indicated otherwise, the assay mixture for ketose reductase contained 100 mM potassium phosphate (pH 7.0), 0.25 mM NAD(P)H, 0.3 M fructose and whitefly enzyme. The assay mixture for SDH contained 100 mM Tricine-KOH (pH 8.5), 2.5 mM $NAD(P)^+$, 0.1 M sorbitol and whitefly enzyme. Maximal activities and K_M values were determined by non-linear regression analysis of the dependence of activity on substrate concentration using the GraFit program (Leatherbarrow, 1992), after correction for no substrate controls. The results presented show the means of triplicate assays ± S.E.M.

2.4. Antibody production and peptide isolation

Ketose reductase, purified through the Matrex Orange step, was precipitated in 80% (v/v) methanol. Following lyophilization, the dried protein pellet was dissolved in 50 mM HEPES-KOH, pH 7.9, containing 2% SDS, 30% sucrose and 0.1% bromophenol blue and then heated to 100°C for 2 min. The 38.7 kD KR polypeptide was separated by electrophoresis in a 1.5 mm 11% SDS–PAGE

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

minigel (Chua, 1980). For antibody production, the gel was stained for 15 min with 0.5% Coomassie Brilliant Blue R-250 in 10% (v/v) acetic acid and 30% (v/v) isopropyl alcohol and destained in 5% (v/v) acetic acid and 16.5% methanol. The stained band corresponding to the KR polypeptide was rinsed in water and used for immunization of rabbits by a commercial service (Cocalico Biologicals, Reamstown, PA). Antibodies against NADPH-KR were affinity purified by adsorption to the KR polypeptide immobilized on PVDF membrane and elution with low pH (Smith and Fisher, 1984).

For peptide sequencing, polypeptides from the SDS–PAGE gel were electrophoretically transferred to Immobilon-P (Millipore, Bedford, MA) PVDF membrane (Bauw et al., 1989). Following transfer, the membrane was rinsed in H_2O, the margins stained with Coomassie Blue and regions containing the KR polypeptide were excised. The membrane pieces containing immobilized KR were either submitted directly for sequencing or digested with cyanogen bromide or trypsin (Fernandez et al., 1994). Cyanogen bromide- and trypsin-generated peptides were isolated by reverse-phase HPLC (Salvucci et al., 1993) and subjected to Edman degradation analysis at the Arizona State University Macromolecular Facility.

2.5. Electrophoresis, Western blot analysis and isoelectric focusing

The gel system of Chua (1980) was used for SDS–PAGE as described above. Non-denaturing PAGE was conducted in 5% polyacrylamide gels using the same system, but without SDS. For Western blot analysis, polypeptides were electrophoretically transferred to Immobilon-P PVDF membrane or nitrocellulose in 50 mM Trizma base/50 mM boric acid (Bauw et al., 1989) for 1 h at 100 V. Following blocking with 4% (w/v) non-fat milk, blots were probed with affinity-purified KR antibody and developed by incubation with an alkaline phosphatase-conjugated secondary antibody (Salvucci et al., 1993). Isoelectric focusing was performed in a vertical polyacrylamide slab gel as described by Gullian et al. (1984). Crude soluble insect extracts were microdialyzed for 3 h against 10 mM HEPES-KOH, pH 7.9, prior to isoelectric focusing in a 0.75 mm 5.5% polyacrylamide gel. Sorbitol dehydrogenase activity was detected by staining for activity as described (Shaw and Prasad, 1970), using $NADP^+$ in place of NAD^+ and 100 mM Tricine-NaOH, pH 8.5, as the buffer. The stained band was excised and polypeptides were separated by SDS–PAGE. The KR polypeptide was detected by Western blot analysis as described above.

2.6. Miscellaneous

Protein concentration in whitefly extracts was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Crude soluble insect extracts were prepared by extraction in 50 mM HEPES-KOH, pH 7.9, and centrifugation at 14 000***g*** for 10 min. The apparent molecular mass of whitefly KR was determined by gel filtration chromatography on a Sephacryl S-300 column as described above. The apparent molecular weight of the KR polypeptide was determined by densitiometric analysis of the SDS–PAGE gels (Salvucci et al., 1993) after calibration against molecular weight standards (Bio-Rad, Hercules, CA).

3. Results

In a previous study we found that extracts prepared from adult silverleaf whiteflies catalyzed NADPH-dependent reduction of fructose to sorbitol (Wolfe et al., 1998). These same extracts also catalyzed the reverse reaction, i.e. $NADP^+$-dependent oxidation of sorbitol to fructose. Since enzymatic rates were fastest in the direction of sorbitol synthesis and net synthesis of sorbitol occurs in heat stressed whiteflies, we will refer to the enzyme in whiteflies as an NADPH-KR for its physiological function.

The NADPH-KR from adult whiteflies was purified over 1400-fold to apparent homogeneity (Table 1, Fig. 1). The most effective step in the procedure was affinity chromatography on a dye-ligand column. Whitefly KR was soluble and had an apparent subunit molecular weight of 38.7 kD on SDS–PAGE (Fig. 1). Dithiothreitol and 2-mercaptoethanol inhibited the activity of the enzyme in both directions (data not shown). The molecular mass of the KR holoenzyme was approximately 132 000 as determined by chromatography on a Sephacryl S-300 column (data not shown). Thus, the active enzyme is probably tetrameric. The isolectric point of the native enzyme was approximately 5.64.

The reactions catalyzed by NADPH-dependent aldose reductase and NAD^+-dependent sorbitol dehydrogenase are readily reversible (Jeffery and Jörnvall, 1988). Similarly, NADPH-KR purified from whiteflies also catalyzed $NADP^+$-dependent sorbitol oxidation. However, as has been shown for NAD^+-SDHs (Jeffery and Jörnvall, 1988), the pH optima of the two reactions were very different (Fig. 2). Ketose reduction catalyzed by isolated whitefly KR had a pH optimum of 7.0, similar to the measured pH of the insect hemolymph (c.f. Mack and Vanderberg, 1978). In contrast, the pH optimum for sorbitol oxidation was greater than 9.0.

Purified whitefly KR exhibited an almost exclusive requirement for NADPH for ketose reduction. The activity with NADH was $< 2.8\%$ of the activity with NADPH (data not shown), which may merely reflect contamination of the NADH by a small amount of NADPH. The apparent K_M(NADPH) for ketose reduction was 9.8 ± 2.5 μM, whereas the apparent K_M ($NADP^+$) for sorbitol oxidation was 57.9 ± 7 μM.

Table 1
Purification of NADPH-KR from adult silverleaf whiteflies

Purification step	Activity (IU)	Protein (mg)	Specific activity[a] (IU mg protein $^{-1}$)	Recovery (%)	Purification (-fold)
Crude homogenate	91.1	533.7	0.17	100	1
Supernatant	74.7	379.3	0.20	82	1.2
Anion-exchange	62.9	120	0.52	69	3.1
Ammonium sulfate	60.8	59	1.0	67	5.9
Gel-filtration	38.2	26.5	1.4	42	8.2
Dye-ligand affinity	30.9	0.2	154.5	34	909
Cation-exchange	12	0.05	240	13	1412

[a]NADPH-dependent ketose reduction was measured with 340 mM fructose.

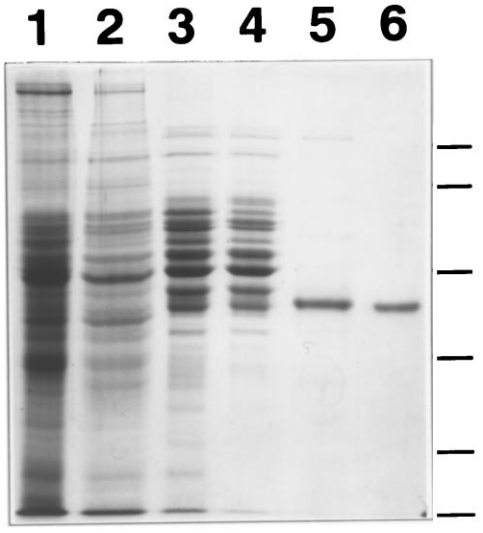

Fig. 1. Polypeptide profiles corresponding to the various steps in the purification of whitefly NADPH-KR. Polypeptides were separated by SDS–PAGE and visualized by staining with Coomassie Blue. The lanes correspond to polypeptides in the crude extract (lane 1), crude supernatant (lane 2) and after chromatography through the Q-Sepharose (lane 3), Sephacryl S-300 (lane 4), Matrex Orange (lane 5) and Poros-SP cation exchange (lane 6) steps. The lines on the right of the figure indicate the positions of molecular weight standards for 97.4, 66, 45, 31, 21 and 14.4 kD.

A most unusual feature of whitefly KR was its extremely low affinity for fructose. The purified enzyme exhibited an apparent K_M for fructose of 619 ± 91 mM (Fig. 3). A similar apparent K_M was determined for the enzyme in crude whitefly extracts (data not shown). The affinity for sorbitol was much higher; the apparent K_M for sorbitol was 21 ± 2.5 mM. Near their respective pH optima, the maximal KR activity of the purified whitefly enzyme was 16-fold higher than the maximal SDH activity.

Since sorbitol accumulates in the silverleaf whitefly upon exposure to elevated temperatures, we determined the temperature optima for ketose reduction and sorbitol oxidation by the purified whitefly KR. Near their respective pH optima, both activities increased with temperature from 10°C to 45°C with an overall Q_{10} of about

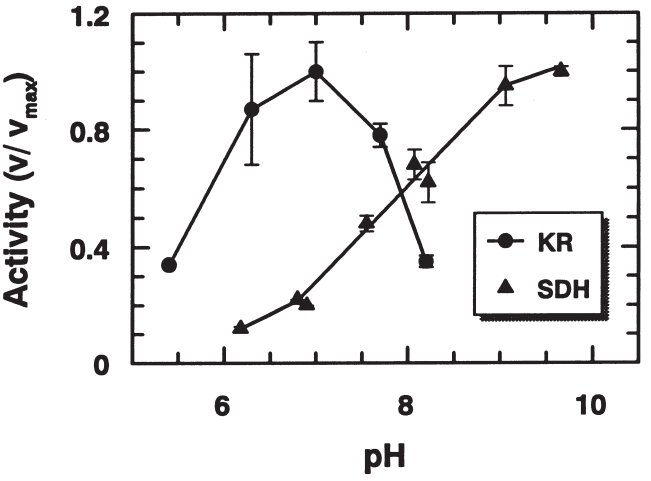

Fig. 2. The effect of pH on the relative activities of whitefly NADPH-KR. Ketose reductase (●) and sorbitol dehydrogenase (▲) activities were measured at the indicated pH. The pH ranges were obtained with 100 mM of the following buffers: potassium phosphate, pH 5.4–8.2, sodium acetate, pH 5.9–6.4, and glycine-NaOH, pH 6.4–9.66. Activity, v/v_{max} , is the rate at a given pH divided by the rate at the optimal pH.

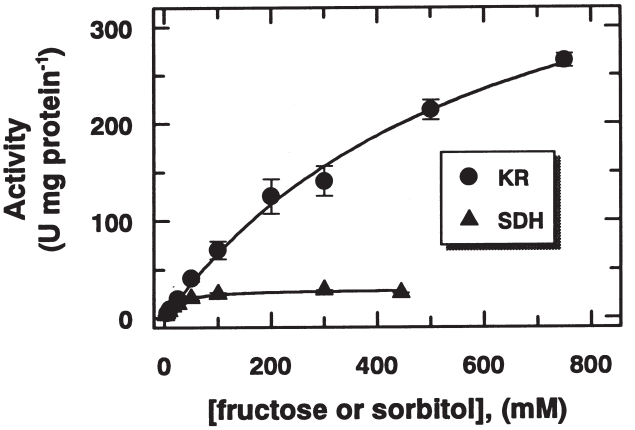

Fig. 3. The effect of substrate concentration on the activities of purified whitefly NADPH-KR. Ketose reductase (●) and sorbitol dehydrogenase (▲) activities were measured in the presence of the indicated concentrations of fructose or sorbitol, respectively, at either pH 7 (ketose reductase) or 8.5 (sorbitol dehydrogenase).

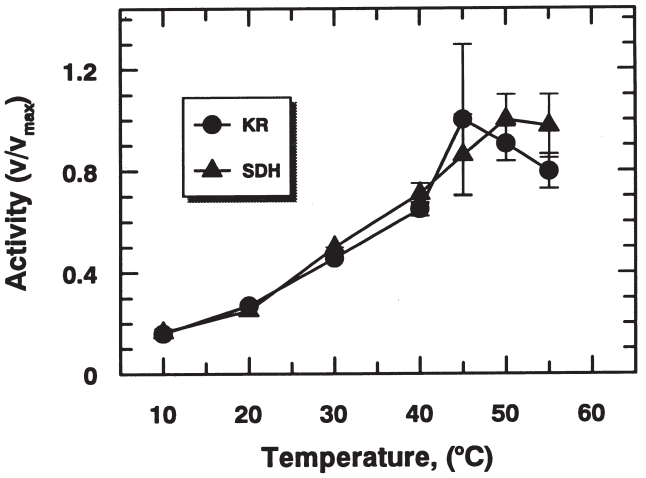

Fig. 4. The effect of temperature on the relative activities of purified whitefly NADPH-KR. Ketose reductase (●) and sorbitol dehydrogenase (▲) activities were measured at the indicated temperatures at pH 7 or 8.5, respectively. Activity, v/v_{max} , is the rate at a given temperature divided by the rate at the optimal temperature.

1.4 (Fig. 4). The temperature optimum for sorbitol oxidation was 50°C, whereas the optimum for ketose reduction was 45°C. Although there was some decrease in activity above 50°C, KR and SDH activities were still higher at all temperatures between 40°C and 55°C than at temperatures $<$ 40°C.

Three separate attempts to obtain the amino acid sequence of the whitefly NADPH-KR by N-terminal sequencing of the polypeptide were unsuccessful, indicating that the N-terminus of the NADPH-KR is probably modified. To circumvent this problem, cyanogen bromide and tryptic peptides were isolated from the 38.7 kD NADPH-KR polypeptide and subjected to Edman degradation analysis. Table 2 shows the amino acid sequences of one of the tryptic and two of the cyanogen bromide peptides. When subjected to BLAST analysis, all three peptides corresponded to regions in the published sequences of sheep liver (Table 2) and other NAD^+-SDHs.

Antibodies generated against purified whitefly NADPH-KR recognized a 38.7 kD polypeptide on Western blots of crude whitefly extracts and purified whitefly KR (Fig. 5). To verify the specificity of the antibody and the identity of the 38.7 kD polypeptide as NADPH-KR, crude extracts from adult whiteflies were also fractionated by non-denaturing isoelectric focusing and the gels stained for $NADP^+$-dependent SDH activity. When the stained doublet from isoelectric focusing was electrophoresed on a SDS-polyacrylamide gel, the antibody recognized a 38.7 kD on a Western blots (data not shown). In addition, the antibody recognized a band on Western blots of non-denaturing PAGE gels that corresponded to the SDH activity band (data not shown).

Immunoreactive bands corresponding to the 38.7 kD NADPH-KR were also detected on Western blots of

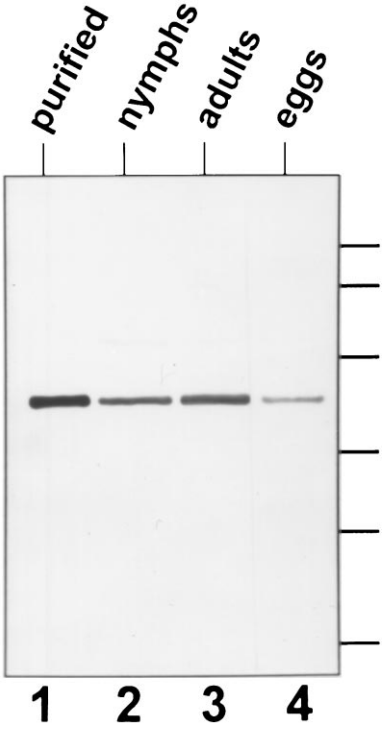

Fig. 5. Occurrence of NADPH-KR in whiteflies at various stages of development. Antibodies against purified NADPH-KR were used to probe Western blots of purified whitefly NADPH-KR (lane 1) and polypeptides in soluble extracts of third and fourth instar whitefly nymphs (lane 2), adult whiteflies (lane 3) and whitefly eggs (lane 4). The lines on the right of the figure indicate the positions of molecular weight standards for 97.4, 66, 45, 31, 21 and 14.4 kD.

Table 2
Amino acid sequence of tryptic and cyanogen bromide (CNBr) peptides from whitefly NADPH-KR and the corresponding sequence in sheep liver NAD^+-SDH (Accession No. 898831). Identical residues between the two sequences are underlined

Peptide	Whitefly NADPH-KR[a]	Sheep liver NAD^+-SDH[b]
1. (CNBr)	M-VIGHEAGGTV	^{63}MVLGHEASGTV
2. (tryptic)	R/K-YNLTPMLTF	^{107}RYNLSPTIFF
3. (CNBr)	M-VTVPLVNA	^{276}MTSVPLVHA

[a]Assignment of the first residue is based on the site of cleavage of cyanogen bromide and trypsin.
[b]The numbers refer to the position of the first residue of the peptide in the known sequence (Jörnvall et al., 1995).

third and fourth instar nymphs and whitefly eggs (Fig. 5). Measurement of enzyme activity in extracts prepared from nymphs and eggs showed that these extracts were capable of catalyzing rates of NADPH-dependent fructose reduction equivalent on a soluble protein basis to extracts prepared from adult whiteflies (data not shown).

4. Discussion

The conventional route of sorbitol biosynthesis in most animals including insects involves reduction of glucose by an NADPH-dependent aldose reductase (Jeffery and Jörnvall, 1988). However, there was little or no detectable aldose reductase activity in extracts of the silverleaf whitefly, despite the fact that this insect accumulates considerable amounts of sorbitol when exposed to elevated temperatures (Wolfe et al., 1998). Instead the predominant activity in the whiteflies was that of an NADPH-KR which converted fructose to sorbitol. In the present study, purification of the whitefly NADPH-KR facilitated detailed characterization of the enzyme. Most importantly, the amino acid sequences of internal peptides from the enzyme definitively established that the whitefly enzyme is related to NAD^+-SDH and not to the NADPH-dependent aldose reductases.

In addition to the similarity in primary structure, the subunit and holoenzyme molecular masses of the whitefly NADPH-KR resembled those reported for other NAD^+-SDHs (Jeffery and Jörnvall, 1988). These enzymes are tetrameric zinc containing enzymes that are structurally similar to alcohol dehydrogenase and other members of the medium-chain dehydrogenase/reductase family (Jörnvall et al., 1987, 1995). Sorbitol dehydrogenases are generally thought to be involved in sorbitol catabolism, in contrast to NADPH-dependent aldose reductases which are generally responsible for sorbitol synthesis (Jeffery and Jörnvall, 1988). Aldose reductases share almost no homology with NAD^+-SDHs. Instead, they are monomeric enzymes that are structurally similar to aldehyde reductase and other members of the aldo-keto reductase superfamily (Bohren et al., 1989).

The coenzyme preference of the whitefly KR differs from all other SDHs that have been thoroughly characterized (Ng et al., 1992; Jeffery et al., 1984; Karlsson et al., 1989), including the cold-inducible enzyme found in *Bombyx* eggs (Yaginuma and Yamishita, 1979; Niimi et al., 1993). Interestingly, an $NADP^+$-dependent SDH activity was one of three distinct SDHs identified in *Drosophila melanogaster* (Bischoff, 1976). Unfortunately, the hexose product of this activity was not characterized. An $NADP^+$-dependent SDH was also reported in silkworm eggs, but its product, glucose, indicated that this enzyme is an aldose reductase (Yaginuma and Yamishita, 1979). NADPH-dependent ketose reductase activity was reported in *E. solidaginis*, but the activity was only about a third of the activity of the NADPH aldose reductase and the reduced product was not characterized (Storey and Storey, 1981). Thus, our investigations of polyol enzymes in silverleaf whiteflies are the first to document the occurrence of an enzyme that uses NADPH as the coenzyme (Wolfe et al., 1998), but is structurally similar to SDH (Table 2). This enzyme occurred in an active form in adult and juvenile whiteflies and in eggs. The intracellular location of the NADPH-KR in whiteflies is currently unknown, but presumably it is localized in a way that facilitates sorbitol accumulation in the hemolymph.

NAD^+-dependent sorbitol dehydrogenases generally have low affinities for their substrates (Jeffery and Jörnvall, 1988). Even so, the affinity of the whitefly NADPH-KR for its ketose and polyol substrates was remarkably low and the Vmax was very high compared with NAD^+-SDHs. For example, the K_M(sorbitol) and (fructose) values for the whitefly enzyme were both four-fold higher than for the sheep liver NAD^+-SDH (Jeffery and Jörnvall, 1988), while the Vmax was 30-fold higher than the *Bacillus subtilis* enzyme (Ng et al., 1992). Although very low, the affinity of the whitefly NADPH-KR for fructose (i.e. K_M = 619 mM) was similar for crude and purified enzyme. It is likely that the low affinity for fructose is physiological since (1) the plant phloem tissue where this insect feeds can contain sucrose concentrations in excess of 600 mM (Fischer and Gifford, 1986) and (2) whiteflies have considerable α-glucopyranosidase activity for hydrolyzing sucrose to glucose plus fructose (Salvucci et al., 1997). Thus, the availability of high concentrations of sucrose, together with a high maximal velocity of ketose reduction and favorable intracellular $NADPH/NADP^+$ ratios (unpublished data), makes sorbitol biosynthesis from fructose via NADPH-KR kinetically possible in the whitefly.

Most studies of sorbitol metabolism in insects have focused on the role of polyols in cryoprotection (Yaginuma and Yamishita, 1979; Lee and Denlinger, 1991; Storey and Storey, 1992 and references therein). These studies have shown that glucose is mobilized from stored glycogen and then reduced to sorbitol via an NADPH-dependent aldose reductase (Yaginuma and Yamishita, 1979; Joanisse and Storey, 1994). In the whitefly, exposure to elevated temperatures triggers sorbitol biosynthesis from recently ingested sucrose rather than from stored carbohydrate (Wolfe et al., 1998). Both glucose and fructose are made available from sucrose by the action of α-glucopyranosidase (Salvucci et al., 1997), but it is fructose that is the substrate for sorbitol biosynthesis. Since glucose and fructose are interconvertible in their phosphorylated forms, there must be some evolutionary advantage to using fructose for sorbitol biosynthesis. As in cold treated insects (Yaginuma and Yamishita, 1979; Storey and Storey, 1992), pyridine

nucleotide in the form of NADPH is the required coenzyme for sorbitol synthesis in heat stressed whiteflies. To use both fructose and NADPH, whiteflies have evolved an SDH with a different coenzyme requirement than other SDHs that have been described.

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